Distribution of von Willebrand Factor in Porcine Intima Varies with Blood Vessel Type and Location

Jacob H. Rand, Lina Badimon, Ronald E. Gordon, Ronald R. Uson, and Valentin Fuster

The von Willebrand factor (vWF) has been generally accepted as a marker for endothelial cells. In a systematic immunolocalization study of porcine blood vessels that used indirect immunofluorescence with a monospecific polyclonal anti-vWF and two monoclonal anti-vWFs, we observed that vWF is not universally distributed in intact, fresh endothelia. vWF is consistently localized in veins, with the exception of the pulmonic vein. In arteries, vWF is generally absent except for areas of the distal abdominal aorta, the vaso vasorum of the thoracic aorta, and the pulmonic artery. We conclude that there are regional differences in the distribution of vWF in the various endothelial beds of pigs. (Arteriosclerosis 7:287–291, May/June 1987)

The von Willebrand factor (vWF) is a high molecular weight multimeric glycoprotein that promotes platelet adhesion to exposed vascular subendothelium and subsequent platelet thrombus formation. Immunolocalization of vWF has been an accepted criterion for identifying endothelial cells and has been applied for that purpose for the validation of tissue culture systems and for histopathologic diagnosis. There is evidence that vWF may play a role in atherogenesis. Pigs with von Willebrand disease have been found to be resistant to aortic arteriosclerosis. Since it is possible that this resistance may be due to impairment of the platelet interaction with the arterial wall, we planned to investigate whether vWF is uniformly distributed in swine intima in vivo.

In addition, any possible differences in vWF distribution in different blood vessels could be an important factor to consider in further investigations of the platelet-vessel wall interaction in pigs. Using immunolocalization techniques, we found that the distribution of vWF in pigs is not homogeneous throughout the vasculature and differs markedly from that observed in the human vasculature.

Methods

Tissues

The procedures performed in this study were all in accordance with appropriate institutional guidelines and also followed the American Heart Association guidelines for animal research. Three normal pigs and two pigs homozygous for von Willebrand disease (vWF:Ag < 3% in plasma) were used for these studies. Porcine blood was collected into 0.11 M sodium citrate and was spun at 250 g for 15 minutes; then platelet-rich plasma was removed, smeared onto glass slides, and allowed to air dry. Each animal was deeply anesthetized by a combined intramuscular injection of 25 mg/kg of ketamine (Ketalar, Parke Davis, Morris Plains, New Jersey) and 1 mg/kg of xylazine (Rompun, Miles Laboratory, Shawnee, Kansas) and was then intubated. Each animal was then euthanized by an overdose of pentobarbital sodium solution (Fort Dodge Laboratories, Fort Dodge, Iowa). Blood vessels and tissues were collected immediately after sacrifice. The blood vessels were handled as carefully as possible to avoid mechanical or air deendothelialization. Bone marrow was collected by aspiration, then smears were made onto glass slides. Because of the surprising results of pilot studies, which showed no detectable immunofluorescence for vWF in arteries, the tissues (other than bone marrow and platelets) were treated in three different ways before application onto glass slides for immunofluorescence. The treatment included: 1) immediate fixation in 2% paraformaldehyde dissolved in iso-osmolar phosphate buffered saline (PBS), pH 7.1, followed by snap-freezing in methylbutane containing dry ice; 2) immediate snap-freezing in methylbutane containing dry ice; and 3) snap-freezing in liquid nitrogen. The three methods yielded the same results. The tissues were then embedded in O.C.T. compound (Miles Laboratories, Naperville, Illinois); then 2-μm cryostat sections were cut and mounted onto glass slides. The integrity of the endothelium was confirmed by routine histochemical staining and by routine transmission electron microscopy.

Immunolocalization Studies

The studies were performed by indirect immunolocalization using three different primary antibodies. One was a monospecific rabbit anti-human vWF IgG developed as previously described, which cross-reacted with normal porcine plasma by immuno-electrophoresis, but not with plasma from pigs with von Willebrand disease. The other antibodies were two mouse monoclonal antiporcine vWF ascites fluids (kindly provided by David Fass) which were coded W1-5 and W1-8 and have been previously well characterized. To briefly review their properties, both W1-5 and W1-8 recognize porcine vWF in the fluid

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Figure 1. Transmission electron micrograph of porcine pulmonic vein showing an intact endothelial layer. By immunofluorescence (Figure 3C), there is no vWF detectable in the intima of this blood vessel. Bar = 1.5 nm.

Figure 2. A. Megakaryocyte from normal pig bone marrow showing immunofluorescence for vWF. Bar = 8 μm. B. Smear of normal porcine platelet-rich plasma showing granular immunofluorescence for vWF. Bar = 8 μm.

phase. However, W1–5 also recognizes porcine vWF mounted onto polystyrene tubes and cross-reacts with fluid phase human vWF, while W1–8 recognizes neither surface immobilized porcine vWF nor soluble human vWF. Neither of these antibodies inhibits ristocetin-induced platelet aggregation nor the nonristocetin dependent agglutination of human platelets (PAF activity).

Indirect immunofluorescence was performed in the following manner. The sections of tissue were incubated with a 1:100 dilution of rabbit anti-vWF in iso-osmolar phosphate buffered saline (PBS), pH 7.4 for 45 minutes at room temperature; then they were washed in PBS and incubated a second time with FITC-conjugated goat antirabbit IgG (Cappel Laboratories, Cochranville, Pennsylvania) diluted 1:100 in the same buffer. The mouse monoclonal anti-vWF antibodies were used under similar conditions at a titer of 1/5 and were followed by FITC-conjugated goat antimouse IgG (Cappel Laboratories) at a titer of 1/100. Additionally, control experiments were performed utilizing nonimmune rabbit serum and mouse ascites fluid. The sections were viewed and photographed as previously described. Care was taken to ensure the uniformity of photographic exposure conditions for the specimens.

Results

Conventional histochemical staining revealed intact endothelium within the vessels collected. This was confirmed by transmission electron microscopy (Figure 1). All three antibodies, the polyclonal, and the two monoclonal anti-vWFs yielded equivalent immunofluorescence results. A summary of our results is shown in Table 1. The vWF was consistently observed in megakaryocytes and platelets (Figure 2). Also, vWF was consistently present in all veins checked except for the pulmonic veins (Figure 3).

A complicated picture emerged from examination of the arterial system (Figure 4). The intima of the thoracic aorta was negative for vWF, and rarely were immunofluorescent areas found. Among the smaller vessels within the vaso vasorum of the thoracic aorta, both positive and negative vessels were noted. In the distal abdominal aorta, positive areas for vWF were noted in the intima.

The remainder of the systemic arterial tree (including
carotid, coronary, internal mammary, gastric, celiac, femoral, and renal arteries) was virtually entirely negative with only rare spots of immunofluorescence. The intima of the pulmonic artery showed the presence of vWF (Figure 4C). The endocardial surfaces of the right and left heart chambers showed no immunofluorescence for vWF.

Neither pig with homozygous von Willebrand disease showed any immunofluorescence for vWF in any blood vessels or platelets. Controls utilizing nonimmune rabbit immunoglobulin and mouse ascites fluid were also negative (Figure 5).

Figure 3. Normal pig veins. A. Cross-section of mesenteric vein showing a layer of immunofluorescence for vWF in the vascular intima. The lumen of the vessel is in the upper portion of the photomicrograph. Bar = 8 μm. B. Jugular vein with slit-shaped lumen along horizontal center of figure bordered by intima shows immunofluorescence for vWF. Background autofluorescence is present in deeper portions of the blood vessel wall. Bar = 8 μm. C. Pulmonic vein showing no immunofluorescence for vWF. The lumen of the vein occupies the upper portion of the photomicrograph, the intima runs horizontally through the center, and background autofluorescence is seen within the media. Bar = 8 μm.

Figure 4. Normal pig arteries. A. Cross-section of splenic artery showing only background autofluorescence in the internal elastic lamina and the intima above it and immunofluorescence for vWF. The lumen occupies the upper portion and the media is below the autofluorescent internal elastic lamina. This lack of immunofluorescence for vWF was, with the rare exceptions noted, typical of the entire arterial tree examined. Bar = 8 μm. B. Cross-section of distal abdominal aorta showing immunofluorescence for vWF in the intima which appears horizontally along the center. The lumen of the aorta occupies the upper portion. A layer of internal elastic lamina having background autofluorescence is seen between the intima and the bottom border of the figure. Bar = 8 μm. C. Pulmonic artery showing the presence of vWF within its intima. This contrasts with the lack of vWF in most systemic arteries and the lack of vWF in the intima of the pulmonic vein.
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Figure 5. Controls using nonimmune rabbit and mouse immunoglobulins. A. Cross-section jugular vein treated with normal rabbit IgG, followed by FITC-conjugated goat antirabbit immunoglobulins. The intimal border is approximately one-fourth of the distance from the top of the figure. Note the minimal background autofluorescence. Bar = 8 μm. B. Mesenteric vein treated with control mouse ascites fluid followed by FITC-conjugated goat antimouse immunoglobulins. The intimal surface is approximately one-third of the distance from the top of the figure. Note the minimal background autofluorescence in the media of the blood vessel. Bar = 8 μm.

Discussion

Previous studies have shown that vWF is present and synthesized in human and bovine endothelial cells.\textsuperscript{15, 18} Therefore, vWF has been considered a reliable marker for endothelium and has been used on a widespread basis for that purpose.\textsuperscript{5}

Previously, Pederson and Fass were unable to detect vWF in cultured endothelium from normal pigs.\textsuperscript{17} Giddings et al.\textsuperscript{18} reported that porcine veins (including umbilical vein and pulmonary vein) showed immunofluorescence for vWF, while aorta and other arteries appeared to be negative or only weakly positive. In their studies, endothelial cell cultures derived from porcine umbilical vein produced Table 1. Summary of Immunolocalization of vWF in Normal Pig Bone Marrow, Blood, and Vasculature

<table>
<thead>
<tr>
<th>Structure</th>
<th>Localization</th>
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<tbody>
<tr>
<td>Megakaryocytes</td>
<td>positive</td>
</tr>
<tr>
<td>Platelets</td>
<td>positive</td>
</tr>
<tr>
<td>Endocardium</td>
<td>absent in right and left heart chambers</td>
</tr>
<tr>
<td>Aorta</td>
<td>absent in intima except for rare spots; present in the vaso vasorum of some vessels</td>
</tr>
<tr>
<td>Thoracic</td>
<td>present in occasional areas</td>
</tr>
<tr>
<td>Abdominal</td>
<td>negative</td>
</tr>
<tr>
<td>Carotid artery</td>
<td>negative</td>
</tr>
<tr>
<td>Coronary arteries</td>
<td>negative</td>
</tr>
<tr>
<td>Internal mammary artery</td>
<td>negative</td>
</tr>
<tr>
<td>Splenic artery</td>
<td>negative</td>
</tr>
<tr>
<td>Renal artery</td>
<td>negative</td>
</tr>
<tr>
<td>Gastric artery</td>
<td>negative</td>
</tr>
<tr>
<td>Celiac artery</td>
<td>negative</td>
</tr>
<tr>
<td>Femoral artery</td>
<td>negative</td>
</tr>
<tr>
<td>Pulmonic artery</td>
<td>positive</td>
</tr>
<tr>
<td>Vena cavae</td>
<td>positive</td>
</tr>
<tr>
<td>Jugular vein</td>
<td>positive</td>
</tr>
<tr>
<td>Renal vein</td>
<td>positive</td>
</tr>
<tr>
<td>Pulmonic vein</td>
<td>negative</td>
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vWF, whereas those derived from porcine aorta failed to synthesize detectable vWF.

Our own results are much in agreement with those of Giddings et al., although there are some differences. We localized vWF throughout the venous tree except for the pulmonic vein where we found it to be absent. The arterial tree was remarkable for absence of vWF, with the exception of the pulmonic artery and the abdominal aorta. The endocardial surfaces of the right and left heart showed no localization for vWF. Nevertheless, the possibility remains that very low levels of intimal vWF are present but are undetectable by immunofluorescence.

Since we have previously shown that vWF in human blood vessels is present underneath, as well as within, endothelial cells\textsuperscript{10, 14, 19} and since subendothelial vWF supports platelet adhesion in high shear rate conditions,\textsuperscript{20, 21} the results of this study may have significant implications with respect to the platelet-arterial wall interaction. On this basis, one would expect that the bulk of systemic arterial tissue, lacking detectable vWF by immunofluorescence, would not significantly differ from porcine von Willebrand disease arteries with respect to platelet adhesion when the perfusing fluids are similar. With respect to aortic tissues, differences may be expected between thoracic and abdominal aortas on the basis of differences in the presence of vWF.

The localization of vWF in porcine abdominal aorta but not in the other arterial segments is of particular interest to us because arteriosclerosis in pigs occurs predominantly in the distal abdominal aorta.\textsuperscript{6, 7, 8} Also intriguing is the possible relationship between our finding of no detectable vWF in normal swine coronary arteries and the finding of a low frequency of spontaneous coronary atherosclerosis in normal, as well as von Willebrand disease, pigs.\textsuperscript{22} Further studies will be necessary to explore the potential relationship between vWF in this site and its proclivity toward atherosclerosis.

At this time, we cannot offer a definitive explanation for the distribution pattern of vWF. The absence of vWF in the pulmonic vein and in the endothelium of the right heart and
the presence of vWF in the pulmonic artery indicate that the deciding factor(s) with respect to the presence of vWF in the intima must be more complex than simply vessel type, i.e., artery or vein. Among possible influences on the expression of vWF are flow forces, blood pressure, and the state of blood oxygenation.

In conclusion, von Willebrand factor cannot be considered a consistent marker for endothelium. The reasons for the differences we observed are not yet understood. Differences in vascular vWF may have implications with respect to platelet reactivity and susceptibility to atherosclerosis. Further studies are planned to investigate these intriguing questions.

Acknowledgments

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Index Terms: von Willebrand factor • von Willebrand disease • atherosclerosis • endothelium
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